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Original Article

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Impact of experimental trauma and niflumic acid administration on antimicrobials' concentration in serum and mandible of rats

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Abstract

Administration of antibiotics and analgesics in surgery or trauma is of great importance for an effective treatment. Trauma, as stress stimulus, causes alterations in various functions of the organism as well as in drug pharmacokinetics. The aim of this study was to determine the effect of trauma upon the serum and bone levels of the antimicrobial ampicillin and cefapirin, with and without co-administration of a non-steroidal anti-inflammatory analgesic (NSAIDs). Fifty-six male Wistar rats were divided into two groups A (control) and B (experimental). Each group consisted of 4 subgroups (n=7) receiving ampicillin, ampicillin with niflunic acid, cefapirin, and cefapirin with niflunic acid. In group B traumatic injury was performed by incision (7 mm length) in the right cheek. The levels of the antibiotics were estimated by the inhibition zone of *B. subtilis*. An increase in antibiotic levels was observed in group B, being statistically significant only for cefapirin level in the mandible. Upon niflumic acid co-administration a statistically significant rise in serum ampicillin and mandible cefapirin levels was observed in both control and experimental groups (student t-test). It can be concluded that the combination of antibiotics and non-steroid anti-inflammatory drugs (NSAIDs) may enhance the antibacterial drug concentration.

Keywords: Trauma, Ampicillin, Cefapirin, Niflumic Acid

Introduction

Skin and soft tissue wound infections are common and need antibiotic treatment. Two-thirds of surgical patients are under antimicrobial therapy. Trauma or surgical procedures affect drug distribution[1-8]. Some pathophysiological mechanisms affecting drug metabolism may be involved, such as delayed gastric emptying or alterations in blood flow through hepatic vessels[9-11]. In addition surgical wounds act as stress stimulus and interfere with drug protein binding. The surgical patients are debilitated both by the disease and the treatment including anaesthesia and drug administration[12-14].

Patients under trauma usually receive various analgesics, which may interact with other co-administrated medications[15-18]. It has been proven that the co-administration of non-steroid anti-inflammatory drugs (NSAIDs) and antibiotics increase the antibiotic levels[19].

The aim of the study was to investigate:

1. The impact of trauma-induced metabolic changes on rats' serum and mandible antibiotics levels.

2. The alterations of antibiotic levels under simultaneous treatment with NSAIDs.

Ampicillin was chosen since it is a commonly prescribed, well tolerated and cost-effective antibiotic, and cefapirin as a cephalosporin with similar properties. Niflumic acid, in comparison with other NSAIDs or acetaminophen, is not associated with increased risk of severe or mild side reactions and its co-administration with antibiotics seems to be safe[20].

Materials and methods

Fifty-six male Wistar rats (weight 150±5 g) age of 50-60 days, were divided into two groups. Animals were cared for

The authors have no conflict of interest.

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GROUP A: CONTROL (n=28)	A1: Ampicillin (Pentrexyl®)	A2: Ampicillin+Niflumic acid (Niflamol®)	A3: Cefapirin (Cefatrex®)	A4: Cefapirin+Niflumic acid
GPOUP B: EXPERIMENTAL – TRAUMA (n=28)	B1: Ampicillin	B2: Ampicillin+Niflumic acid	B3: Cefapirin	B4: Cefapirin+Niflumic acid

Table 1. Experimental groups (Experiment duration 42h).

	AMPICILLIN	CEFAPIRIN	NIFLUMIC ACID
Dosage	1g/kg/8h i.m.	1g/kg/8h i.m.	14mg/kg/8h p.o.
t1/2[22]	69 min	42 min	2 h
Protein Binding[22]	18%	45%	99%
*Vd[22,23]	21 lt/70 kg	49 lt/70 kg	0.12 l/kg
*Vd (Volume of distribution)			

Table 2. Drugs dosage and properties.

in accordance with the principles of the "Guide for the Care and Use of Experimental animals"[21]. Group A served as controls and Group B as the experimental group.

The animals of Group B were submitted to experimental trauma through the whole length of the cheek, and extending up to the mandibular bone.

Each group consisted of 4 subgroups of 7 animals each, receiving the drugs as indicated in Tables 1 and 2. Niflumic acid was given *per os* (X3 daily) using a trans-esophageal catheter. The administration of drugs was performed in 5 doses totally in order to obtain steady state concentrations in plasma to tissues (ratio: steady state condition plasma/tissue), because it is referred that the steady-state drug concentration is usually achieved after three to five half-lives of a given drug. Loading doses circumvent the necessity of waiting three to five half-lives to achieve a maximum therapeutic effect[22]. The choice of the administered doses was made according to studies, suggesting that small animals should be receiving lower doses because of different volumes of distribution[23-25]. The antibiotics were administered intramuscularly every 8 hours. After 2 hours of the last drug administration the animals were sacrificed by decapitation. Plasma was obtained and the hemi-mandible was isolated under sterile conditions.

The concentration of both antibiotics was estimated by the Bennett method of inhibition zone of *B. subtilis* in agar infusion in *Petri* dishes[26]. The bones were incubated in 3 ml of 0.9% NaCl for 24 hours at 8°C. The incubation liquid was infused similarly to serum in *Petri* dishes and the inhibition zone of *B. subtilis* was measured.

The levels of serum free fatty acids (FFA) were determined by the colorometric technique (kit Wako NEFA-C, Wako Pure Chemicals Industries, Osaka, Japan)[27].

The weight of adrenals was estimated as a stress index, since it is known that adrenal hyperplasia under stress conditions is due to over release of corticosteroids[28,29]. Statistical analysis was performed by student t-test.

Results

1) Trauma caused a statistically non-significant ($P<0.10$) increase in subgroup B1, but a statistically significant ($P<0.001$) increase in serum ampicillin levels in subgroup B2, compared to subgroups A1 and A2, respectively (Table 3A).

Niflumic acid led to a statistically significant ($P<0.001$) increase in ampicillin serum levels in treated (received niflumic acid) and untreated animals (A1v A2 and B1v B2) (Table 3A).

Serum cefapirin levels were enhanced statistically significantly ($P<0.01$) only in subgroup B4, but not in A4 ($P<0.10$), as compared to B3 and A3, respectively (Table 3B).

Niflumic acid induced a statistically significant ($P<0.01$) increase in cefapirin serum levels in treated but not in untreated animals (A3v B3 and A4v B4) (Table 3B).

2) Ampicillin levels in the mandible of subgroups B1 and B2 were not statistically significantly higher ($P<0.10$) than those of A1 and A2, respectively (Table 3A).

Niflumic acid did not induce a statistically significant ($P<0.10$) increase in ampicillin mandible levels between treated and untreated animals (A1vA2 and B1v B2) (Table 3A).

Cefapirin mandible levels showed a statistically significant ($P<0.001$) rise in B3 and B4 as compared to A3 and A4, respectively (Table 3B).

Niflumic acid induced a statistically very significant ($P<0.001$) increase in cefapirin mandible levels in both treated and untreated animals (A3vA4 and B3vB4) (Table 3B).

3) The adrenal weight was higher in the experimental group (0.850 ± 0.27mg) as compared to control (0.206 ± 0.12 mg). Free fatty acid (FFA) levels demonstrated a significant increase under the influence of trauma (0.944 ± 0.25 μEq/L) as compared to control (0.290 ± 0.07 μEq/L) (Table 4).

Discussion

The present study demonstrates that trauma can alter the levels of antibiotics in co-administration with niflumic acid.

A. Ampicillin		
Group Drugs	Serum	Mandible
A1 ampicillin	28.5±1.55 μg/ml	19±3.05 μg/g
B1 ampicillin+trauma	30±1.3 μg/ml	22.16±1.85 μg/g
A2 ampicillin+niflumic acid	36±1.68 μg/ml**A1/A2	19.5±1.19 μg/g
B2 ampicillin+niflumic acid+trauma	60.5±4.91 g/ml**A2/B2**B1/B2	20.5±0.65 μg/g
B. Cefapirin		
A3 cefapirin	11.5±2.54 μg/ml	0.93±0.015 μg/g
B3 cefapirin+trauma	14±0.3 μg/ml	1.12±0.07 μg/g**A3/B3
A4 cefapirin+niflumic acid	13.01±1.78 μg/ml	1,56±0,005 μg/g**A3/A4
B4 cefapirin+niflumic acid+trauma	17±0.7 μg/ml*A4/B4 *B3/B4	1.87±0.03μg/g**A4/B4**B3/B4
*p<0.01, **p<0.001		

Table 3 (A+B). Antibiotic levels in serum and mandible after steady state condition obtained via 5 dose drug administration.

Trauma and surgery cause considerable changes in pathophysiological metabolic procedures due to energy demand[30-32]. Blood flow through various organs is affected, gastrointestinal mobility diminishes, the absorption rate is decreased and hepatic metabolism and excretion is impaired[33].

The major source of energy, during the post-operative period, appears to be the body fat stores. Fat is mobilised and transported mainly as FFA bound to serum albumin[34].

Immediately after trauma, FFA concentration is found to be increased[35].

FFA affect the binding of acidic antimicrobials to serum albumin perhaps through competition for binding sites. This leads to increases in unbound drug in serum. So the observed rise in antibiotics levels under trauma may be due to FFA elevation[36].

The observed increase of antimicrobial agents in bone of the experimental group may be due to the enhancement of the free fraction of antibiotic in the extravascular space due to increased distribution[37].

The change in distribution takes place partly because the surplus of the free fraction diffuses into the extravascular space and partly because the bound fraction accompanies the albumin, which, after trauma or surgery, is distributed to the extravascular space to a greater extent. In trauma situations there is an oedema, leading to decreased serum albumin. It is probably a hypovolaemia. The enhancement of cefapirin levels in the mandible of the trauma group could be due to the extravascular diffusion of the albumin- antibiotic complex[38].

It must be noted that plasma albumin binds to a variety of lipophilic molecules such as steroids, hormones and phytochemicals[39]. Albumin is reduced after trauma and corticosterone, as an acute phase hormone, is increased. Then corticosterone may occupy more binding sites in the albumin molecule, and so more free antimicrobial drug is attributed to blood circulation. In addition, the rise in adrenal weight may assert that stress induces changes in corticosteroids release, which is also involved in the protein binding competition[40-42].

The co-administration of the ampicillin with niflumic acid led to a high increase in its serum levels, because niflumic acid, as several NSAIDs, can displace the antibiotic from its binding sites in albumin or tissue proteins. It is already reported in previous studies that NSAIDs can enhance the free fraction of warfarin or clonidine through their displacement capacity from tissues or plasma proteins[43,44]. Our results are in agreement with other investigators who observed similar increases of penicillin in the presence of phenylbutazone[45]. In addition, the levels of niflumic acid in serum following trauma were adequate to occupy the binding sites of albumin, thus displacing the antibiotic and further increasing its serum levels. In contrast, the co-administration of cefapirin with niflumic acid did not increase the serum level of the antibiotic, possibly due to its high binding ability to albumin and to its rapid elimination time (t1/2=42 min) as compared to ampicillin (t1/2=69 min)[22].

	Control	Trauma
Adrenal weight(g)	0.206±0.12	0.850±0.27*
FFA (μEq/L)	0.290±0.07	0.944±0.25*
*p<0.001		

Table 4. Changes of adrenal weight and serum free fatty acid level.

From the previously reported results, it is revealed that the concurrent administration of antimicrobials and non-steroid anti-inflammatory drugs may potentiate the antibiotic concentration leading to a probable favourable clinical therapeutic efficacy of the drugs. However this enhancement may lead to the development of undesirable and/or toxic effects.

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